Phase I Final Report

Innovative Thin Film Fluorescence Sensor Providing Greatly Enhanced Signal-to-Noise and Integrated Solid State Detection. Suitable for Incorporation in Microchip Sensor Systems.

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A unique corrugated-thin-metal-film structure suitable for integration with semiconductor photodetectors, is investigated. For fluorescent spectral monitoring, this structure provides a number of important detection features including fluorescence enhancement for molecules located near the surface of the sensor, excitation wavelength shielding and narrow bandpass filtering of detected wavelengths. Surface dielectrics with chemical or biological-specific properties may also be incorporated into the sensor for additional selectivity.

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With the awareness of chemical and biological terrorism, there is an increasing need from both the public and military for compact, sensitive and sophisticated detection capabilities. This awareness has also led to an increase in the scope of environmental and military species to be monitored. While laboratory based instruments, e.g. gas and liquid chromatographs, mass spectrometers etc., meet the sensitivity requirements they are either not compatible with the process conditions or are unable to sample in restricted areas. What is required are inexpensive, autonomous point monitors - essentially sensors on a chip -, which are compatible with harsh process conditions. These sensors must be rugged, consume low power and be capable of programmed and commanded wakeup. At the same time it is highly desirable that they have the flexibility to be adapted to a variety of applications and monitoring conditions.

Systems & Processes Engineering Corporation (SPEC) and Dr. Michael F. Becker of the University of Texas at Austin have developed and demonstrated a method and process to fabricate a corrugated multiple layer thin metal film to yield a fluorescence sensor with greatly enhanced signal-to-noise. Operation of this sensor relies on the creation of surface plasmons from the interaction of the fluorescent signal with the dielectric-metal interface. Under certain circumstances, the surface plasmons couple across the thin metal surface and subsequently decay via a radiative process, essentially creating a dispersive transmission channel through a nearly opaque metal film. This sensor's unique structure provides both fluorescence enhancement and wavelength filtering. Further, by fabricating the thin film on the face of a semiconductor photodiode, the entire sensor system becomes suitable for incorporation in chip level sensor systems.

From this baseline data, SPEC and Dr. Becker propose to proceed to a Phase II program which will include fabrication of a prototype fluorescence detection system capable of isolating the fluorescence emission of interest from an intelligent polymer film or biological active layer ligated to the corrugated surface. We have developed a number of detection scenarios for this technology. One such DoD application is envisioned in Figure 1. The fluorescence detection system in com-

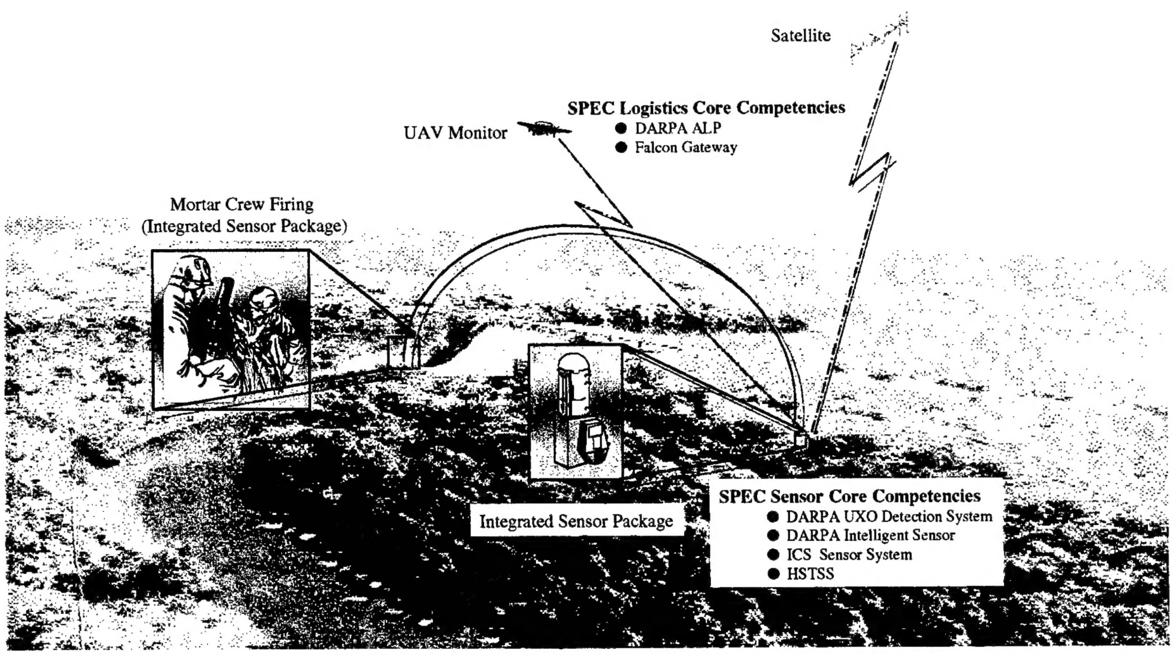


Figure 1 - Envisioned DoD detection system application

bination with a intelligent polymer layer or biological active film, can be used to detect chemical or biological warfare agents. A number of chemical and biological sensing films have been developed including a platinum 1,2-enedithiolate complex that fluoresces at room temperature upon exposure to selected phosphate esters. Organophosphates, fluoro- and cyanophosphates are major constituents of chemical warfare agents.[1] Another detection method to be investigated will be a polymerase chain reaction (PCR) system utilizing molecular beacon technology. This detection methodology is carried out using direct hybridization-based detection of target nucleic acids. These molecular beacons are composed of two fluorophores, a reporter dye and a quencher dye moieties. When bound to the target, the probe is unfolded and the quencher is spatially removed from the reporter dye.[2] By attaching the molecular beacon molecules directly to the corrugated thin film, a very simple and highly sensitive detection system can be developed. Both detection scenarios are ideally suited for the corrugated thin film technology which will provide increased Signal-to-Noise, highly specific analyte detection, estimated 2-3 order increase in fluorescence intensity due to surface related fluorescence enhancement, shielding from background/fluorescence filtering, and fluorescence bandpass tailored by choice of dielectrics and corrugation periodicity.

The envisioned detection system will be designed to withstand the shock of landing in a remote area. SPEC also possesses other core technologies that can be leveraged to provide a complete solution to the development of a deployable sensor package for remote and autonomous military and civilian chemical or biological sensing applications.

SPEC and Dr. Becker have extensive experience implementing lifetime and steady-state fluorescence measurement systems and developing intelligent films and biological receptors. Dr. Becker has extensive experience developing corrugated structures and has a wide range of expertise in developing characterization methodology for these structures. In addition to Dr. Becker SPEC has reached an agreement with Dr. Russell Gruhlke of Breault Research to provide consulting services. Dr. Gruhlke's graduate work involved the development and characterization of these thin structures and his experience and expertise with these structures is unmatched. It is expected that Dr. Gruhlke's input should greatly facilitate the construction of optimized structures.

In this Phase I effort, SPEC and Dr. Becker have successfully demonstrated the construction of a corrugated thin film structure that clearly exhibited fluorescence resulting from surface plasmon coupling across a conducting interface. Excellent control of the period of the structure was obtained and diagnostic indicators of efficient surface plasmon coupling were also developed.

SPEC has manufactured, demonstrated, and delivered a number of biological and chemical detection and monitoring systems to NASA Langley, US Army Missile Command, and the Naval Air Warfare Center (NAWC), Aircraft Division at Patuxent River. SPEC is currently under contract with NASA Langley and Lockheed Martin to develop a Distributed Strain Sensor (DTS) for health monitoring of cryogenic tanks in the next generation space shuttle, the X33. This program follows the successful completion of a space certified Distributed Temperature Sensor (DTS) which was recently delivered on time to NASA for integration into an experimental flight of the X-33. SPEC is also currently manufacturing a handheld fluorosensor and a frequency-domain lifetime fluorescence instrument for use in biological warfare detection and biomedical diagnostic applications. Other SPEC sensor customers include the U.S. Air Force Armstrong Laboratory, U.S. Army Chemical Biological Defense Command (CBDCOM), and NASA Langley. This unique capability and experience provides for quick response design, assembly, and testing of highly

sophisticated electronics ensuring successful completion of integration and certification of shock hardened, flight qualified sensor systems.

SPEC is in the process of establishing partnerships for the successful completion of the Phase II program and to further the development of spin-off technologies. SPEC has also aggressively pursued the development of potential markets with end users of this technology. During Phase II, SPEC will continue the aggressive development of these potential partnerships and commercial prospects including a detailed Phase III commercialization plan.

Along with a 13 year track record of innovation in government funded research and development, SPEC has performed R&D and has manufactured electronic components for private industry. SPEC has developed strategic partnerships and joint ventures with corporations such as 3M, Siemens and Morningstar Diagnostics to ensure the commercial success of technologies developed by SPEC. In recognition of this success, SPEC was among 62 companies and individuals who were presented with the 1998 Tibbetts Award from the Small Business Administration (SBA) in Wastington DC. The Tibbetts Award is SBA's annual recognition given to 'models of technology excellence.' SPEC has also successfully launched a spin-off company, Extreme Devices in 1998. SPEC has received a letter of intent for $4.5 Million dollars to finance this venture.

1.0 Phase I Research Objectives and Accomplishments

In this Phase I effort SPEC and Dr. Becker successfully demonstrated the construction of a corrugated thin film structure that clearly exhibited fluorescence resulting from surface plasmon coupling across a conducting interface. Excellent control of the period of the structure was obtained and diagnostic indicators of efficient surface plasmon coupling were also developed. Building upon these accomplishments, SPEC has developed a follow-on, Phase II plan for further refinement and development of the integrated sensor technology. This aggressive plan takes advantage of on-going technology advances and uses SPEC laboratory resources to provide a near term system for testing and evaluation by DoD personnel. The Phase I research program was oriented toward accomplishing the following objectives;

- Develop corrugated thin-film structures,
- Etch grating structure into substrate,
- Deposit controlled-thickness layer of metal onto grating,
- Deposit layer of dielectric on top of metal film,
- Develop measurement protocols,
- Identify suitable chemical/biological dielectrics,
- Investigate possibility of integrated sensor/control/data acquisition, and
- Phase II prototype development plan

1.1 Phase I Result Summary

SPEC has successfully completed all the key technical objectives described in the Phase I proposal. With completion of this Phase I program, SPEC has developed a follow-on Phase II program plan. The following sections provides a brief summary of conclusions and Phase II system recommendations.

1.1.1 Sample Fabrication

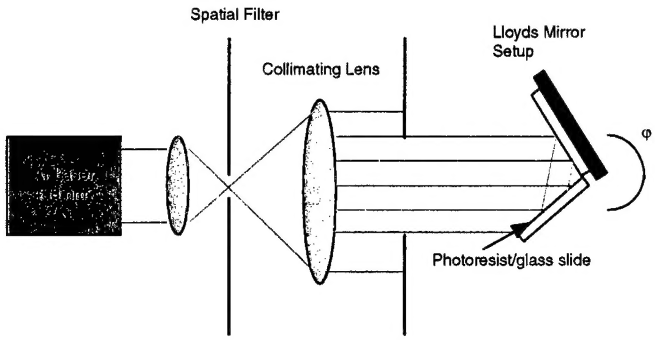


Figure 2 - Lloyds mirror apparatus. The laser interferes at the surface of the photoresist producing a periodic structure whose period Λ is related to the orientation φ by the relation

$$\text{Sin } \varphi = \lambda/2\Lambda$$

In the early stages of evaluation the process used to create the grating structure must be flexible enough to produce gratings with a wide range of periodicity's. NASA Lewis Space Flight Center (LSFC) has recently done some work in this area, employing a Lloyds mirror arrangement (Figure 2) to produce corrugated structures in a photoresist. In this arrangement a spatially filtered Argon ion laser beam is allowed to interfere at the surface of a glass slide with a thin coat of photoresist. The interference creates a standing wave pattern whose periodicity can be varied by rotating the apparatus. To evaluate the properties of thin film structures the photoresist was used as both a substrate to grow the corrugated film structure and to provide a luminescent test material. This was accomplished by only partially developing and then baking the photoresist. This created a corrugated substrate upon which a Ag layer was deposited, resulting in an asymmetric thin film structure with dielectrics of air and photoresist.The photoresist could then be photoexcited and the resulting fluorescence monitored on the far side of the Ag film.

For the initial studies, photoresist and developing solutions were obtained from AZ -photoresist (AZ 1518) and developer AZ 400K. This resist has an absorbance of roughly 0.1 at the Argon ion laser wavelength used to form the standing wave pattern (l = 457.9 nm). Using the Lloyd's mirror arrangement with the mirror adjusted to 13° from normal a grating with a periodicity of 1 mm was prepared. Using a constant laser exposure of ~2.5 mW/cm2, a series of samples were exposed for different duration's ranging from a few seconds to 2 mins. The exposure intervals were necessary to calibrate the depth of the corrugation with exposure time. Twenty second development times in a 1:5 solution of deionized water/AZ 400K developer were used. Silver mirror layers were then deposited onto the thin film structures by vapor deposition and monitored using an in situ quartz crystal microbalance. The thickness of the silver films was further verified using a Tencor Alpha Step 2000 profilometer. An additional attempt to measure the depth of the grating was made using the profilometer, however the period is too short for the lowest sampling rate of the instrument.

Figure 3 shows a scanning electron micrograph of a 3 minute exposed sample of photoresist on glass, coated with 500 Å of silver. In addition an AFM trace of a similar grating is shown in the accompanying figure. The period of the corrugation is ~ 1 μm. This particular sample has an amplitude that is much larger than the desired 25 nm for the final device structure. Nonetheless, it

demonstrates the feasibility of controlling the period of the grating using the Lloyd's mirror technique.

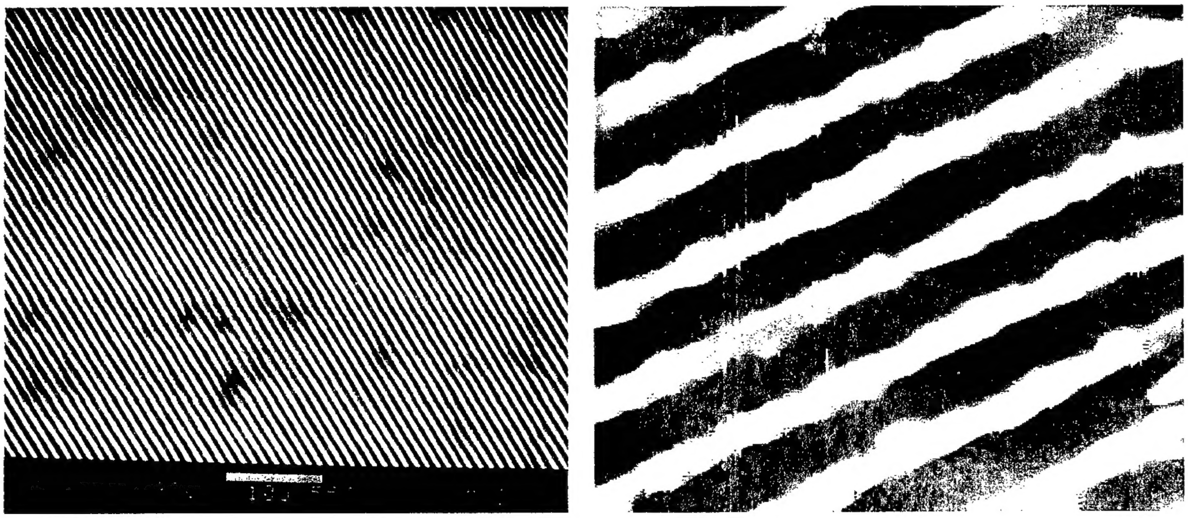


Figure 3 - On the left is a scanning electron micrograph of a grating etched into a silver-coated photoresist pattern on glass. On the right is an AFM of a similar grating. The depth is several 100 nm.

The efficiency of the surface plasmon coupling is extremely sensitive to the depth of the grating. SPEC has developed several diagnostics that indicate the region where surface plasmon cross coupling occurs. In the figure below the large features resembling parentheses were determined to be excellent indicators of the correct grating amplitude. In this diagnostic surface plasmons on the silver grating are directly excited by a helium-neon laser. Subsequent reflection and re-radiation occur with the patterns appearing on a white screen situated at an angle off the incident face of the grating. These diagnostic features are quite sensitive to the depth of the grating, too shallow and they don't appear at all (this can occur even when a first order diffraction spot is visible), too deep a grating and the features become less distinct with light filling in the black regions.

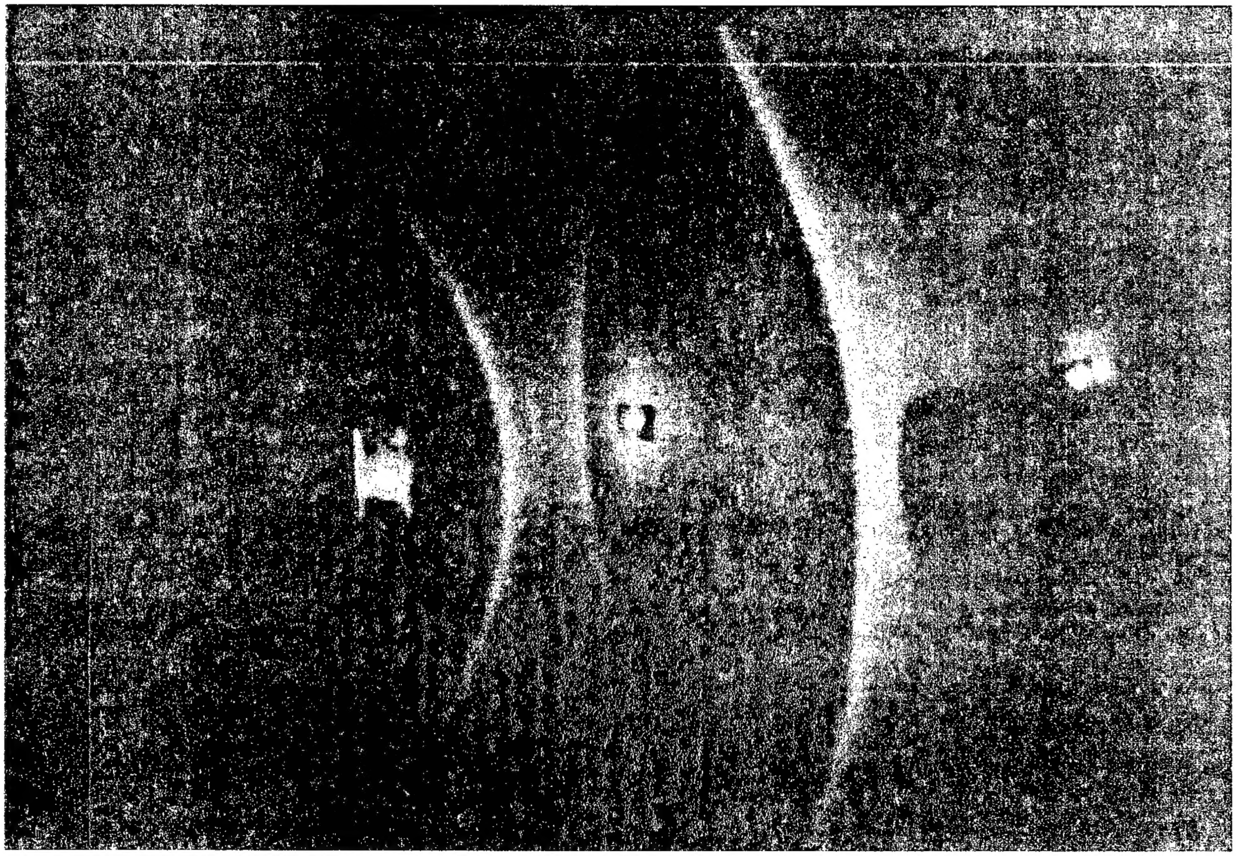


Figure 4 - In this photograph the laser is to the right center and the thin film the bright area to the left center. The screen is in the background intercepting the diffracted and re-radiated light. The screen shows the first and second order diffraction spots with the associated parentheses like features indicative of surface plasmon coupling.

1.1.2 Thin-Film Transmission

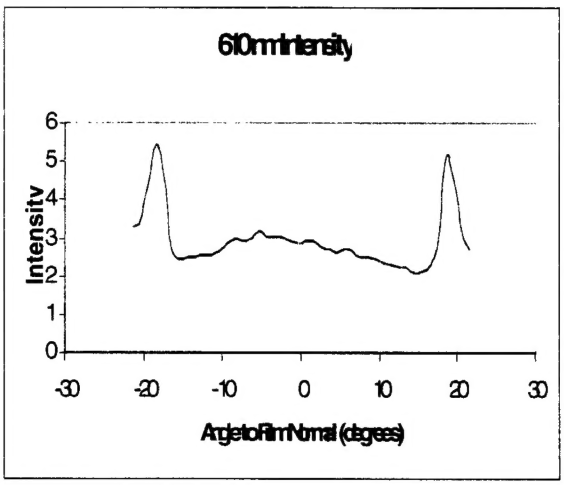
Transmission characteristics of fabricated thin film structures were tested by mounting the glass slide on a rotation stage, exciting the photoresist from the glass side of the structure and monitoring the resulting emission from the silvered face. An air-cooled Argon ion laser (488 nm) supplied the excitation. It was coupled into a fiber optic whose opposite end was mounted on the rotation stage normal to the glass slide. Excitation intensities were ~ 2mW. Emission from the silvered face of the thin film was monitored by a monochrometer equipped with a photomultiplier tube and angular resolution of the emission was obtained by suitably arranged slits and a lens system. Angular resolution was ~0.3 degrees and the monochrometer adjusted for ~2 nm resolution.

Photoresist emits over a broad region ranging from 550-750 nm The close proximity of the thin film allows a relaxation mode via the creation of surface plasmons (SP's). Surface plasmons on the photoresist/Ag interface may subsequently couple to surface plasmons on the Ag/air interface which in turn may radiate over a narrow angular range governed by the equation

$$\mathrm{Ksp} = \frac{2\pi}{\Lambda} + \frac{2\pi}{\lambda}\sin\Theta$$

Here theta is the angle of emission relative to the thin film normal.

This angular dependence is illustrated in the left graph in Figure 5. In this figure the monochrometer was set for 610 nm and the intensity monitored as a function of angle. The sharp angular dependence is typical of surface plasmon emission.Spectra of this emission were also obtained and are shown to the right in Figure 5. Overlaid on this data is a photoresist spectra illustrating the bandpass characteristics of the emission.



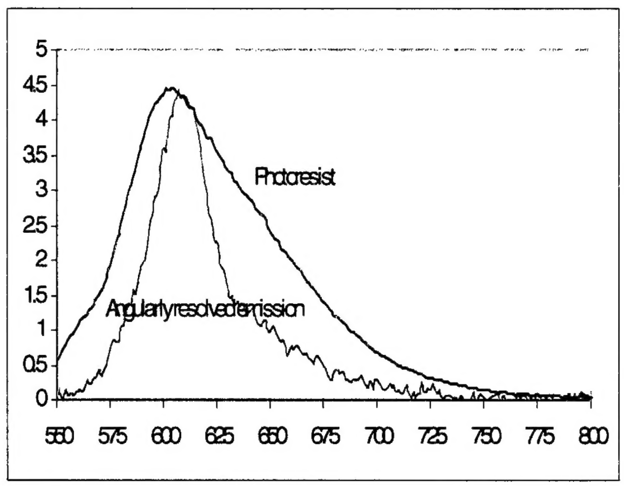


Figure 5 - The graph on the right shows the angular dependence of 610 nm emission. On the left is a spectra of the associated peak

The emission spectra show a strong peak overlaid on some background emission. The silver film is not completely opaque to incident radiation and the background emission mimicking the photoresist spectra is the fraction of normal fluorescence transmitted directly through the silver. To isolate the surface plasmon related fluorescence, which is TM polarized, a polarizer was placed in front of the spectrometer and spectra obtained with the polarizer at two orientations, 90 degrees apart. The data is shown in Figure 6. The sample employed had a silver coating of 40 nm. Since cross coupling of surface plasmons should be relatively efficient up to ~100 nm, increasing the Ag thickness should make effects of background fluorescence negligible.

Spectra at a range of angles were also taken and the results summarized in Figure 7. The spectral width of the surface plasmon related radiation is determined by the spread in the dispersion relations. SP coupling only occurs when the condition that the difference in the wavenumbers of the two surface plasmon modes is equal to a multiple of $2\pi/\Lambda$. Surface roughness, variations in dielectric constant and non-uniformity of the periodic structure all contribute to this spread. Some parallel work on thin film sensors has indicated that spectral half widths of 40-50 nm can be achieved.

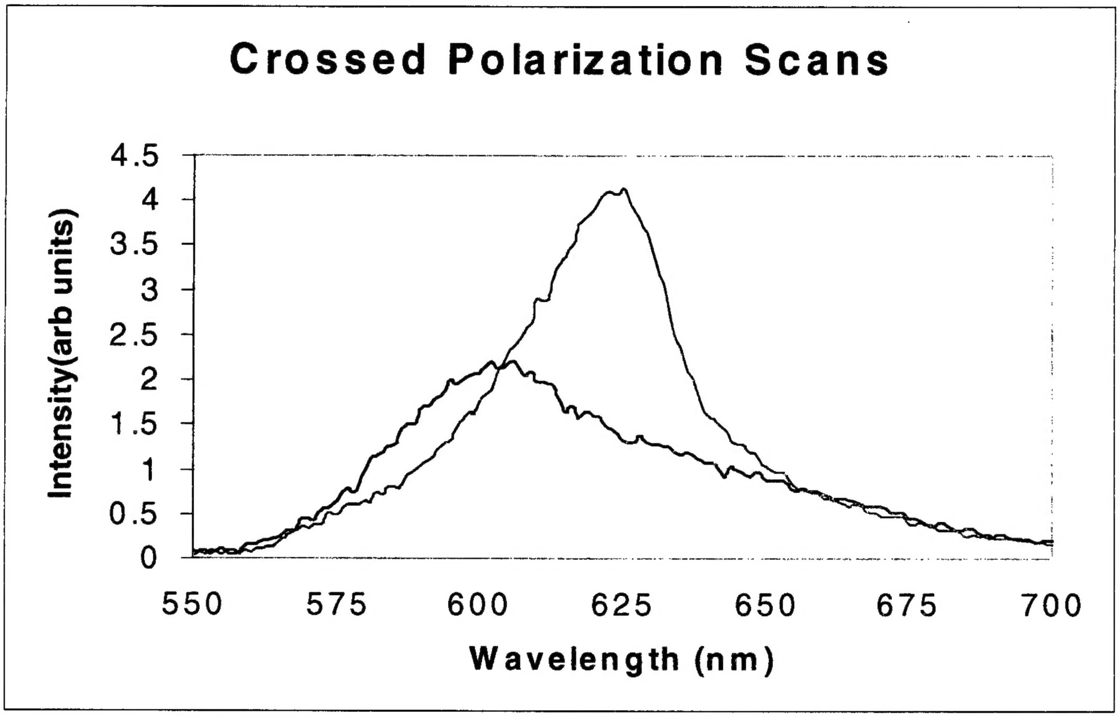


Figure 6 - Spectra of emission centered at 18.5 degrees with two perpendicular orientations of a polarizer in front of the spectrometer

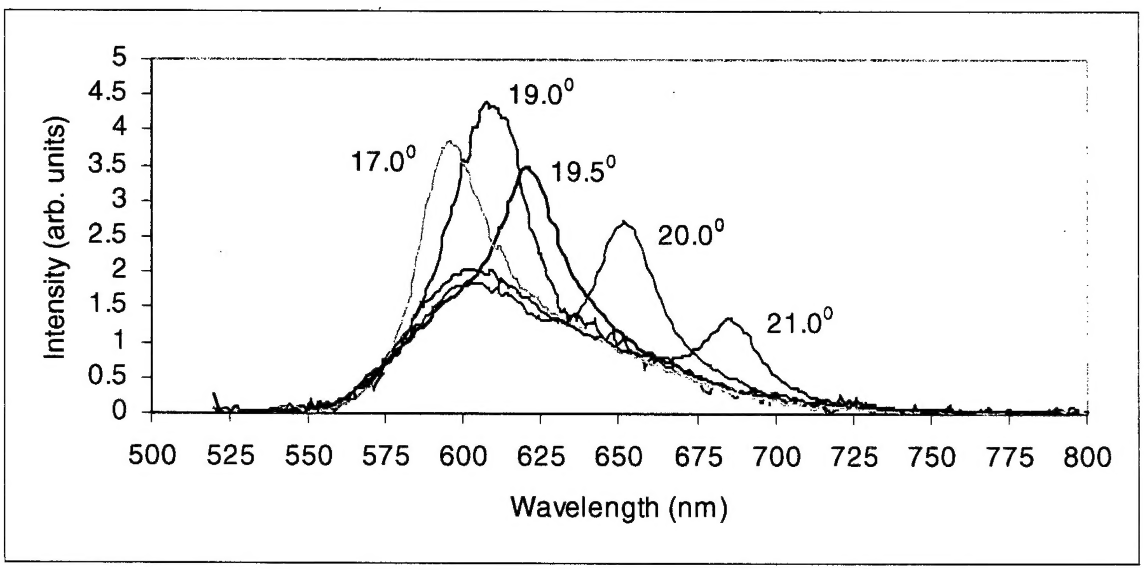


Figure 7 - Spectra taken at a range of angles from the film normal.

1.1.3 Surface Specific Dielectrics

SPEC has recently contacted Dr. Andrew McGill of the Naval Research Laboratory and Dr. Tim Swager of M.I.T. regarding the integration of surface specific polymers into the thin film structure. NRL has extensive experience developing analyte specific thin films, particularly for SAW device applications. They currently have a suite of custom and commercial polymers specific to a wide range of analytes. Some of these polymers are specific to single analytes, others to general classes of chemicals. NRL has developed a method involving pulsed laser deposition, which supplies very fine control (nm) of the thickness. This method has been developed under DARPA funds and is suitable not only for the proposed thin film sensor but also for the construction of sensor arrays as well.

M.I.T. has also done extensive work on analyte specific coatings. Most recently they, in partnership with Nomadics Inc., successfully demonstrated an instrument for the detection of land mines. This instrument employed a polymer specifically tailored for TNT. Dr. Swager has also indicated they have good control of surface thickness in the deposition process.

In recent conversations both M.I.T. and N.R.L. have voiced their willingness to support further efforts on the fabrication of an analyte specific device involving the thin film structure.

2.0 Sensor Concepts Utilizing the Corrugated Thin-Film Technology

Chemical or biological detection is based on measuring fluorescence resulting from interactions of polymer films or biological receptor molecules with a target of interest. In some cases the excitation may also excite other fluorophores which emit at different bands. In this case it would be possible to distinguish, using bandpass filtering effect of the corrugated thin film, the source and intensity of the various fluorescence.

In Phase II, SPEC and Dr. Becker will investigate and test a platinum 1,2-enedithiolate complex that fluoresces at room temperature upon exposure to selected phosphate esters. Organophosphates, fluoro- and cyanophosphates are major constituents of chemical warfare agents. SPEC proposes to coat a corrugated thin film with a candidate platinum 1,2-enedithiolate complex which become fluorescent upon exposure to a nerve gas simulant.

Another Phase II detection method to be investigated will be a polymerase chain reaction (PCR) system utilizing molecular beacon technology. This detection methodology is carried out using direct hybridization-based detection of target nucleic acids. These molecular beacons are composed of two fluorophores, a reporter dye and a quencher dye moieties. The fluorescence is quenched when self hybridized due to the close (<70 Å) spatial proximity of the reporter dye and the quencher dye moieties. When bound to the target, the probe is unfolded and the quencher is spatially removed from the reporter dye. By attaching the molecular beacon molecules directly to the corrugated thin film, a very simple and highly sensitive detection system can be developed. Both detection scenarios are ideally suited for the corrugated thin film technology which will provides increased Signal-to-Noise, highly specific analyte detection, estimated 2-3 order increase in fluorescence intensity due to surface related fluorescence enhancement, shielding from background/fluorescence filtering, and fluorescence bandpass can be tailored by choice of dielectrics and corrugation periodicity.

Figure 8 is a schematic of the integrated sensor utilizing chemical or biological sensitive films. The entire system is compact and highly sensitive to the biological of chemical target of interest.

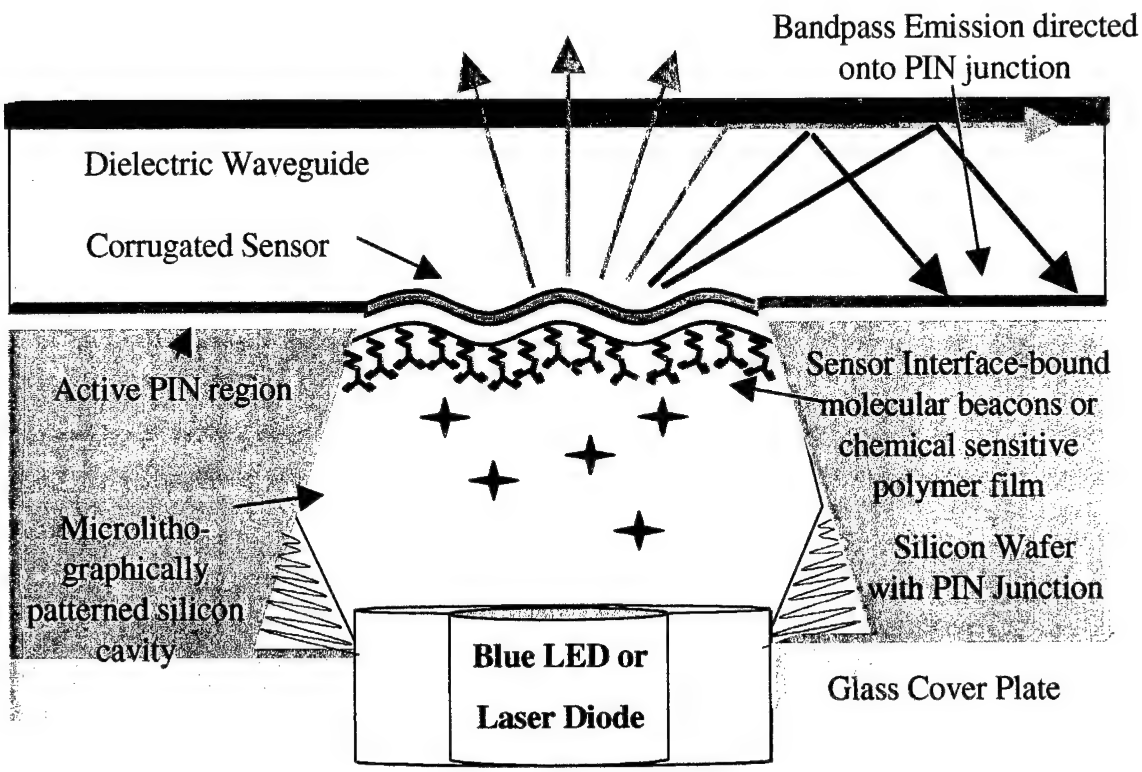


Figure 8 - Schematic of integrated sensor system with various detection scenarios. Stars represents targets of interest.

2.1 Chemical Sensitive Fluorescent Polymer Layers

Recent results by Pilate and coworkers have demonstrated a way to rapidly and selectively detect volatile fluoro- and cyanophosphates, the major constituents of chemical warfare agents. The method relies on a platinum 1,2-enedithiolate complex with an appended alcohol that fluoresces at room temperature upon exposure to selected phosphate esters.[1]

By exciting at 450 nm and monitoring the 605 and 710 nm emissions, Pilate was able to show detection of volatile phosphate esters using an immobilized heterocyclic-substituted platinum 1,2-enedithiolate with an appended alcohol as a sensor molecule. Figure 9 is the luminescence spectra of a typical exposure of the film.[1]

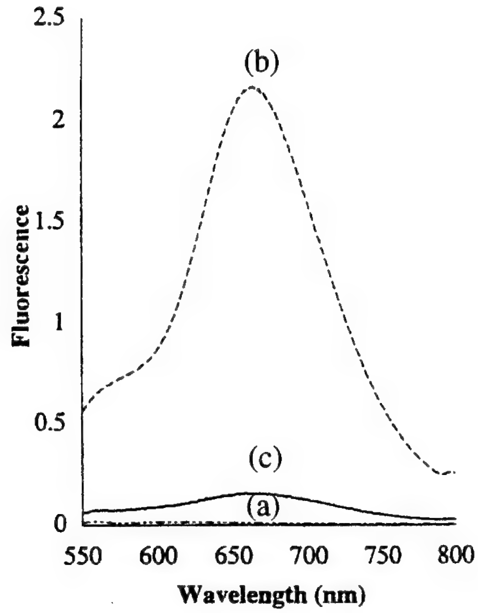


Figure 9 - The luminescence spectra of platinum 1,2 -enedithiolate complex immobilized in a cellulose acetate film. (a) Control film. (b) Film exposed to 0.9 g/m3 $OP(OEt)_2Cl$ in N_2 for 2 minutes at 50 mL/s. (c) Film exposed to HCl.[1]

2.2 Biological Warfare Detection Using Dye Labelled Nucleic Acids (Molecular Beacons)

The measurement of biological targets or events within biological targets has undergone a quantum leap in utility as a result of the application of molecular biological tools. Specifically nucleic acid hybridization allows the direct measurement of target genes or the product of their expression, mRNA. Enhanced sensitivity has been realized with the discovery of genetic amplification methods most notably the polymerase chain reaction.[17 - 18] The specificity, engendered in the hybridization between two complementary nucleic acids, coupled to the extreme sensitivity of the polymerase chain reaction has resulted in a wide variety of assays to measure a myriad of different biological phenomena. For example, general assays to detect total bacterial load or the presence of a specific pathogen have been developed. Qualitative or quantitative measures of gene expression have been established and used to determine the efficacy of therapeutic treatments. Surveys for literally hundreds of potential mutations within a given gene have been carried out using nucleic acid-based assays.

A polymerase chain reaction (PCR) detection system utilizing molecular beacon technology is ideally suited for detection of biological targets of interest by the corrugated thin film technology. The design and synthesis of a series of molecular beacons was carried out using direct hybridization-based detection of target nucleic acids. These molecular beacons are quenched when self hybridized due to the close (<70 Å) spatial proximity of the reporter dye and the quencher dye moieties. When bound to the target, the probe is unfolded and the quencher is spatially removed from the reporter dye. Another PCR based fluorescence technology is 5' nuclease probes which using Taq polymerase. Taq polymerase undergoes endonuclease activity during the course of nucleic

acid amplification and will hydrolyze an oligonucleotide sequences only when it is hybridized to the target and only if the 3' end is phosphorylated. The hydrolysis of the dual dye-labeled oligonucleotide sequence releases the reporter dye into solution with a concomitant increase in fluorescence emission.

Two different nucleic acid-based chemistries can be exploited in the Phase II proposal, molecular beacons and 5' nuclease probes. Molecular beacons were coined in a report by Tyagi and Kramer[2] and describes a format where an intact probe is self-quenched due to the proximal location of a reporter and a quencher fluorescent dye. When bound to the target these two dyes are separated and quenching is relieved. In contrast 5' nuclease assays exploit the endonuclease activity of most thermal stable DNA polymerases to cleave a dual labeled oligonucleotide probe. Upon hydrolysis a reporter dye is released and its fluorescence intensity increases dramatically.[19] Both of these assays have their own advantages and disadvantages. Each however, offers unique applicability where sensitivity juxtaposed with simplicity is desired.

A schematic representation of the combined PCR/fluorogenic probe methodology is shown in Figure 10. The methodology is based on the denaturation of a target strand of DNA/RNA followed by reannealing with a fluorogenic labeled strand of a DNA/RNA probe sequence and subsequent extension during Taq polymerase activity. Two distinct nucleic acid chemistries will be explored, both of which have as the basis for their detection a reduction in the quenching of the intact probe molecule when the target template is present. In both formats, a fluorescent reporter dye covalently linked to an oligonucleotide probe is quenched by a second dye molecule also covalently attached to the same probe. The release of quenching, due to either the unfolding of the probe (Figure 10a) or the hydrolysis of the probe (Figure 10b), is measured by fluorimetry. The increase in fluorescence is quantitative for the initial template concentration.

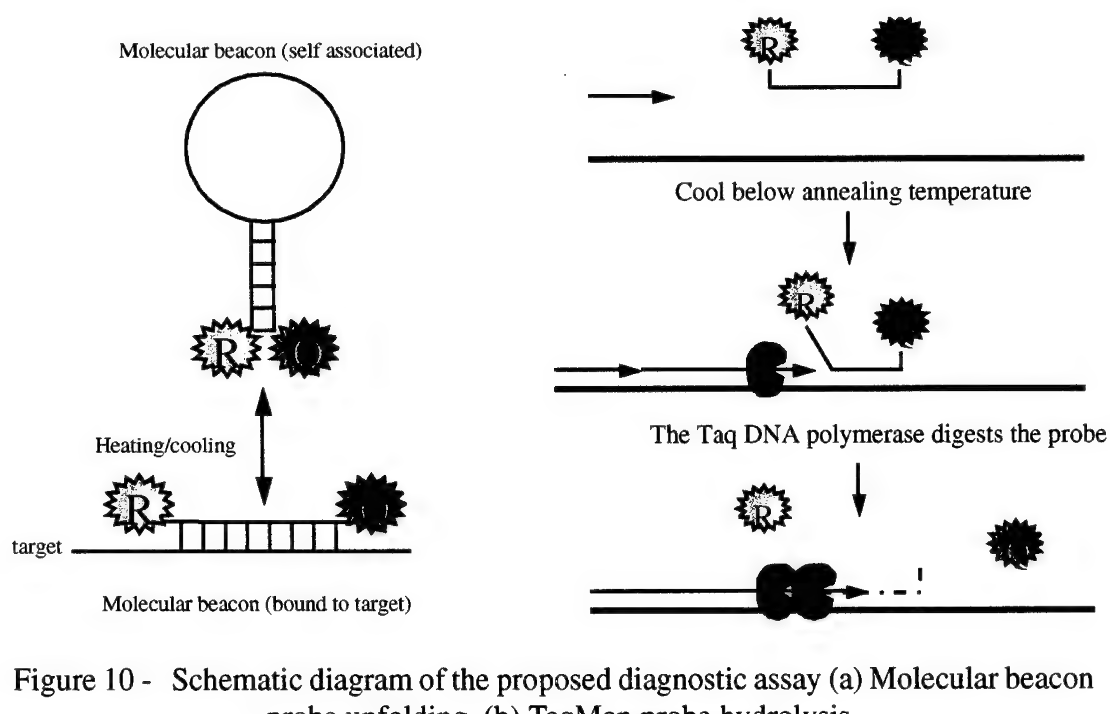


Figure 10 - Schematic diagram of the proposed diagnostic assay (a) Molecular beacon probe unfolding, (b) TaqMan probe hydrolysis

Two model nucleic acid targets will be explored with PCR-based assays in Phase II. The human ß-actin gene was used as the first target and one of the hypervariable regions of the 16S rRNA was used as the second target. The human ß-actin gene was chosen since it represents a generic target that, with some sequence variation, is found in all higher eukaryotes. It is commonly employed as an internal control since its levels of expression are constant in most cell types. The human ß-actin was used as a model for subsequent systems that test, for example, the effect of various environments on human body function. In a reverse transcriptase-5' nuclease assay mode, the expression level of any individual gene can be followed. As part of the Phase II objectives, RT-5' nuclease assays will be performed in a manner similar to DNA/RNA-based PCR methods in terms of their quantitative nature.

The 16S rRNA is conserved in all eubacteria and serves as basis for taxonomical discrimination between genera and species. PCR based methods that probe the 16S rRNA have been developed and these can be used as the basis for developing a diagnostic assay for virtually any bacteria. In general operation, it can be used to follow bacterial contamination and in more specific interrogation, it can be used to detect and quantify any particular bacteria. Therefore, the course of specific diseases or other bacterial processes can be monitored. The performance of each of these assays with their respective targets will be evaluated based on specificity and sensitivity.

3.0 Phase II recommendations

With the successful completion of the Phase I feasibility demonstration, SPEC has developed a follow-on Phase II prototype development program. SPEC will continue to work with Dr. Michael Becker and has a working agreement with Dr. Russell Gruhlke for the development and optimization of corrugated thin film technology. Dr. Gruhlke performed his graduate work on these thin film devices and his expertise in this area is unmatched. Dr. Gruhlke has agreed to provide consultation services with the aim of rapidly optimizing the thin film structures and providing production methodologies to allow for repeatability and quality control.

At the end of Phase II, SPEC will deliver a fully operational chemical or biological sensor based on the corrugated thin-film technology. In order to deliver this prototype sensor system, SPEC has identified the following key technical tasks;

Task 1: Optimize Bandpass Charateristics

Successful completion of a sensor prototype requires that we increase control of the bandpass characteristics. Specific problems which likely contribute to increased bandwidth include:

- Surface roughness,
- irregularities in the periodicity of the corrugated structure, and
- variations in the local dielectric constant

AFM and SEM pictures indicate that control of the periodocity is well in hand. Surface roughness and variations in the local dielectric constant, however must be addressed. The use of photoresist, which undergoes a physical change upon exposure to UV light, likely contributes to variations in the local dielectric constant. The photoresist is typically underexposed and underdeveloped to obtain a smoothly varying surface. SPEC plans to employ a RIE etch process to create the corrugation pattern directly in the cap oxide of a photodetector. This process uses a specific etchant gas to etch the photoresist down to the cap oxide layer, which is not touched in the process.

The gas is then changed to one which will etch the cap oxide but leave the unexposed photoresist unharmed. The use of this process should alleviate any problems associated with changes in local dielectric constant. Similarly, the RIE process should result in a smoother surface with more uniform depths. While there are a number of parameters to vary in this process, SPEC believes we can rapidly narrow these into an acceptable operating range. Dr. Russell Gruhlke will also help in this effort. His experience in the operating parameters and efficienies of optimized thin film structures should allow us to rapidly zero in on suitable production parameters.

Task 2: Characterize Fluorescence Enhancement Features

In addition to bandpass transmission characteristics, fluorescence enhancement features must also be characterized. Again the addition of Dr. Gruhlke to the team should help facilitate this effort. Fluorescence enhancement has a tremendous effect on the final sensitivity and specificity of the instrument. Fluorescecne of molecules near the upper surface is expected to be enhanced 2-3 orders of magnitude. The resulting large signal allows increased filtering from background and results in excellent signal to noise. The fact that only molecules near the upper surface-specific dielectric exhibit enhanced fluorescence greatly increases the specificity of the device. SPEC, Drs. Becker and Gruhlke will investigate the optimum coating thickness for the various dielectrics chosen and develop suitable procedures to control the coating thickness's during production.

Arrays of solid state optical detectors are available and integration of these unique thin film structures with a variety of different dielectrics offers the possibility of detector arrays with tremendously increased analyte specificity.

Task 3: Optimize thin-film thickness

In addition to increased bandpass control, metal coating thickness for filtering of excitation and isotropic emission also need to be optimized. Dr. Gruhlke's experience in the production of thin film structures should greatly facilitate this task. The metal thickness is typically a tradeoff between shielding from background noise and transmitted signal. Too thin a coating and the excitation and stray fluorescence signals become prominant features. Too thick a coating and the fluorescence signal becomes increasingly attenuated. There is some ambiguous literature on the attenuation of the signal as a function on the coating thickness. SPEC, Drs. Becker and Gruhlke will determine the optimum thickness.

Task 4: Develop and integrate thin film structure with a PIN detector

Critical to the integration of these structures is the development of a process to etch the grating into the cap oxide layer of a suitable detector. We have recently received several samples of PIN diodes from Silicon Sensors Inc. Current plans are to spin on a resist coating which will be exposed and developed with methods perfected on the glass slide samples. To produce shallow surface gratings in the cap oxide, Reactive Ion Etching (RIE) will be used to pattern the photoresist down to the device surface. This technique is capable of very high aspect ratios and under certain conditions will etch only the resist and not the oxide layer. Following this procedure the etchant gas will be changed to CF_4, allowing the oxide to be etched without damage to the photoresist. Etch duration and environmental conditions must be carefully controlled to obtain the desired corrugation amplitude.

Task 5: Develop sensing film applications

In Phase II, SPEC proposes to construct a thin film structure on a solid state photodetector with an integrated surface specific dielectric. This will require that SPEC, Drs. Becker and Gruhlke determine the dielectric constant of selected surface specific dielectrics and learn to control production properties such as layer thickness. Once a suitable sensor is constructed SPEC will determine the fluorescence yield and resulting device sensitivity and specificity. SPEC has chosen several possible sensor applications including a vapor phase poison gas detector. However the actual Phase II sensor application will be chosen in consultation with DARPA.

Task 6: Design, Construct and test fully operational sensor package

During the course of the Phase II program SPEC will also determine a number of parameters affecting the final application of the sensor. These include:

- Excitation power requirements.
- Need for a preconcentrator.
- Need to integrate sensor with suitable electronics for excitation, control, data acquisition and transmission.
- Power, data requirements, service life.
- Delivery method, Communication method.

Task 7: Deliver prototype to the Government for test and evaluation

Depending on the application, which will be chosen in consultation with DARPA personnel, SPEC will develop a specific prototype instrument for delivery to the government for testing and evaluation. This instrument will include the sensor, excitation source and sampler. In addition SPEC will consult with DARPA regarding user interface and will deliver a suitable interface with the final prototype.

4.0 Commercialization Plan

Systems & Processes Engineering Corporation (SPEC) provides advanced technology solutions to industry and government customers. SPEC's products include: Gallium Arsenide (GaAs) application-specific integrated circuits (ASICs); computer and telecommunications components and subsystems; high precision/high sensitivity laser based sensor systems for a wide range of applications; and an array of software products, expert systems, and highly specialized cell libraries for designing GaAs ASICs.

Located in Austin, Texas, SPEC is a privately owned business founded in 1986, with a senior engineering staff supported by state-of-the-art equipment and facilities. SPEC specializes in developing technology products which require multi-disciplinary engineering capabilities. In working with its diversified customer base, the company has established a tradition of excellence over a wide range of technologies and applications. SPEC's engineering laboratories consist of modern test equipment and an extensive suite of Computer Aided Design (CAD) tools for the design of GaAs and CMOS ASICs, custom electronics, optical components, and mechanical assemblies.

SPEC collaborates with major universities and research organizations in basic and applied research. As a systems engineering company, our product and customer base is equally divided

between government agencies and major corporations. SPEC's US Government contracts span a broad range of disciplines and agencies within the Department of Defense and NASA, and have provided an important launch pad for the company's commercial product development activities. SPEC's corporate customers and strategic partners include Sun Microsystems, E-Systems, Texas Instruments, Honeywell, 3M, Siemens, and Compass Design Automation.

SPEC has experienced significant growth in the last two years, more than doubling in both manpower and income. SPEC currently has over 80 employees and projected 1999 earnings of $15 Million.

4.1 SPEC's commercialization successes

SPEC is currently under contract with NASA Langley and Lockheed Martin to develop a Distributed Strain Sensor (DTS) for health monitoring of cryogenic tanks in the next generation space shuttle, the X33. This program follows the successful completion of a space certified Distributed Temperature Sensor (DTS) which was recently delivered on time to NASA for integration into an experimental flight of the X-33. This unique capability and experience provides for quick response design, assembly, and testing of highly sophisticated electronics ensuring successful completion of integration and certification of space flight hardened and qualified equipment.

SPEC is in the process of establishing partnerships for the successful completion of this Phase II program and to further the development of spin-off technologies such as an intelligent corrosion indicator in the commercial aircraft industry. SPEC has also aggressively pursued the development of potential markets with end users of this technology. During Phase II, SPEC will continue the aggressive development of these potential partnerships and commercial prospects including a detailed Phase III commercialization plan.

Along with a 13 year track record of innovation in government funded research and development, SPEC has performed R&D and has manufactured electronic components for private industry. SPEC has developed strategic partnerships and joint ventures with corporations such as 3M, Siemens and Morningstar Diagnostics to ensure the commercial success of technologies developed by SPEC. In recognition of this success, SPEC was among 62 companies and individuals who were presented with the 1998 Tibbetts Award from the Small Business Administration (SBA) in Wastington DC. The Tibbetts Award is SBA's annual recognition given to 'models of technology excellence.'

SPEC has also successfully launched a spin-off company, Extreme Devices in 1998. SPEC has received a letter of intent for $4.5 Million dollars to finance this venture. This demonstrates the company's belief in the technology and our commitment to its development and introduction to U.S. industry.

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